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SEROPREVALENCE OF HEPATITIS C VIRUS IN THE GENERAL POPULATION OF NORTHWEST TANZANIA

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Abstract. Sera from 516 participants enrolled in a population-based cross-sectional study in northwest Tanzania were tested for antibodies to hepatitis C virus (HCV). The mean age of study subjects was 29 years (range = 16–49 years); 43% were men, 6% reported a history of blood transfusion, and 4% were infected with human immunodeficiency virus-1 (HIV-1). Although 53 of 516 sera (10.3%, 95% confidence interval [CI] = 7.8–13.2%) were repeatedly reactive by a third-generation enzyme immunoassay (EIA-3), only 6 of the 53 were positive when tested with a third-generation recombinant immunoblot assay (confirmed HCV seroprevalence = 1.2%, 95% CI = 0.4–2.5%). The positive predictive value of the HCV EIA-3 in this population was 18.8% (95% CI = 7.0–36.4%). False positivity was not correlated with EIA-3 optical density values, age, sex, infection with HIV-1, or a history of blood transfusion, but it was marginally associated with increased serum IgG levels. We conclude that the prevalence of HCV is low in this region and that the HCV EIA-3 has a higher false-positivity rate in this population than has been reported among U.S. blood donors.

Hepatitis C virus (HCV) is the major etiologic agent of post-transfusional hepatitis worldwide[1] and may also be an important cause of community acquired non-A, non-B hepatitis in certain parts of Africa.[2,3] Infection with HCV is usually asymptomatic, but may result in chronic hepatitis with progression to cirrhosis and hepatocellular carcinoma.[4,5]

Assays for the detection of antibodies against HCV proteins were developed soon after the viral genome was cloned in 1989.[6] An enzyme immunoassay (EIA) represented an important advance in reducing HCV transmission through blood transfusion. Supplemental tests for detecting antibodies to HCV, such as the recombinant immunoblot assay (RIBA), have been developed to confirm reactivity detected by the EIA.[7]

Studies of volunteer blood donors and general populations have shown considerable geographic variation in HCV seroprevalence:[8] 0.5–1.5% in western Europe, northern Europe, North America, and Australia; 1.5–2.5% in Japan and the Mediterranean region; and as high as 14% in Egypt. In sub-Saharan Africa, data on the prevalence of HCV are limited and the extent of HCV infection remains unknown in most countries. In this study, we estimated the seroprevalence of HCV in a population-based cross-sectional study in northwest Tanzania.

METHODS

During 1989 and 1990, a population-based study was conducted to estimate the prevalence of human immunodeficiency virus (HIV) in northwest Tanzania. The protocol for this study was approved by the appropriate review committees of the Tanzanian Ministry of Health and the National Cancer Institute. The study methods have been described elsewhere.[9] Briefly, the target population consisted of people living in rural, peri-urban (mainly subsistence farmers and their families), and urban areas (commercial workers). Subjects 15–49 years of age were randomly selected from household rosters supplied by the local government. Prior to interviewing subjects, pre-HIV test counseling was performed and informed consent was obtained. With a participation rate of 57% for the urban and peri-urban populations and 90% for the rural population, 4,086 consenting subjects were interviewed and a venous blood sample was collected. Sera were separated from whole blood within 8 hr after collection; they were stored in multiple aliquots at −20°C, sent overseas on dry ice, and kept frozen until testing.[9]

To estimate the seroprevalence of HCV in this cohort, 516 of the 2,206 subjects with sufficient archived serum were randomly selected to be representative of the geographic distribution of the population (rural, urban, and peri-urban). Sera were screened for antibodies to HCV with a third-generation EIA (EIA-3; Ortho Diagnostic Systems, Raritan, NJ), which is based on recombinant antigens c22, c200, and NS5. Reactions were determined spectrophotometrically and results were expressed as optical density (OD) of the test sample and compared with the cut-off value obtained as specified by the manufacturer. Samples for which the OD was lower than the cut-off values were considered HCV EIA-3 non-reactive. Sera that resulted in an OD greater than or equal to the cut-off value were considered initially reactive and were retested in duplicate. If the sample OD was equal to or greater than that of the cut-off value in at least 2 of 3 tests, the sample was considered HCV EIA-3 repeatedly reactive.

All HCV EIA-3 repeatedly reactive sera were tested with a third-generation strip immunoblot assay (RIBA-3™; Chiron Corporation, Emeryville, CA). This assay is based on 2 recombinant proteins (c33c and NS5) and 2 synthetic peptides (c100p and c22p). In addition to these HCV antigens, the assay strips contained 2 levels of human IgG as internal controls (Level I, weak positive and Level II, moderate positive), and the human superoxide dismutase (SOD) antigen. The strips were mounted on a white background and each band was scored using a 10 point gray scale, which was then adapted to the scoring criteria outlined in the manufacturer's protocol. Reactivity was determined by comparing the inten-

TABLE 1
Selected demographic variables and hepatitis C virus (HCV) antibody status in northwest Tanzania, 1989–1990

Variables	Total (%)	Anti-HCV status*			P†
		Positive (%)	Indeterminate (%)	Negative	
Total	516	6 (2.5)	21 (8.3)	489	
Sex					
Male	221 (43)	4 (1.8)	10 (4.5)	207	0.23
Female	295 (57)	2 (0.7)	11 (3.7)	282	
Age (years) mean	29.1	32.0	30.2	29.0	0.43
Residence					
Rural	212 (41)	3 (1.4)	6 (2.8)	203	
Peri-urban	92 (18)	1 (1.1)	6 (6.5)	85	0.91
Urban	212 (41)	2 (0.9)	9 (4.3)	201	
History of blood transfusion in the past 5 years					
No	485 (94)	6 (1.2)	21 (4.3)	458	0.52
Yes	31 (6)	0	0	31	
Infection with HIV-1‡					
No	475 (96)	5 (1.1)	17 (3.6)	453	
Yes	22 (4)	1 (4.5)	1 (4.6)	20	0.14

* HCV antibody status is based on testing algorithm using EIA-3 and RIBA-3™. EIA = enzyme immunoassay; RIBA = recombinant immunoblot assay.
† Values shown are for the comparison of the HCV-positve and -negative groups.
‡ Based on 497 subjects. HIV-1 = human immunodeficiency virus-1.

sity of the individual antigen bands with that of the IgG Level I internal control. A RIBA-3™ result was considered positive if there were at least 2 HCV antigen bands (c100p, c33c, c22p, or NS5) reactive with an intensity at least equivalent to the Level I control band; indeterminate if there was a single reactive band; and negative if there were no reactive bands. The SOD band was considered positive if its intensity equaled or exceeded that of the Level I control band.

All RIBA-3™ positive and RIBA-3™ indeterminate sera were tested for detection of HCV-RNA using target amplification by a reverse transcription–polymerase chain reaction (RT-PCR, Amplicor HCV PCR; Roche Molecular Diagnostic Systems, Nutley, NJ). The Amplicor HCV PCR test uses primers from the most conserved 5′ untranslated region of the viral genome and detects HCV RNA at a sensitivity of <100 copies/ml of serum.

To investigate potential sources of HCV EIA-3 false reactivity, we compared the IgG concentration (measured by a nephelometric technique at the Mayo Medical Laboratories, Rochester, MN) among 20 randomly selected EIA-3 repeatedly reactive, but RIBA-3™ negative sera, with that of 20 EIA-3 non-reactive sera. The distribution of EIA-3 OD values was plotted and the mean OD value and the standard deviation were calculated for each RIBA-3™ result group.

The serum specimens had previously been tested for antibodies to HIV during the HIV seroprevalence study. The specimens were initially screened by the HIVCheck Rapid Screen assay (Ortho Diagnostic Systems). Reactive specimens were tested with a Western blot (Dupont, Wilmington, DE); positivity was defined by clear reactivity against p24, gp41, gp120, and gp160 antigens.[9]

The associations between HCV status and covariates were assessed by a two-tailed Fisher's exact or the Student's *t*-tests. P values < 0.05 were considered statistically significant. The STATA statistical software package (version 4.0, 1995; Stata, College Station, TX) was used for analysis.

RESULTS

Table 1 shows selected demographic data for the 516 study subjects. The mean age was 29 years (range = 16–49 years) and 43% were men; 41% resided in rural, 41% in urban, and 18% in peri-urban areas. Six percent of the subjects reported a history of blood transfusion and 4% were infected with HIV-1.

Sixty (12%) of the 516 sera were initially reactive for antibody to HCV with the EIA-3 and 53 (88%) of these were repeatedly reactive when retested. Of the 53 HCV EIA-3 repeatedly reactive sera, 26 were negative on the RIBA-3™, 21 were indeterminate, and 6 were positive. Therefore, the overall RIBA-confirmed seroprevalence of HCV infection in this study population was 1.2% (6 of 516, 95% confidence interval [CI] = 0.4–2.5%). Anti-HCV status was not associated with sex, age, place of residence, previous history of blood transfusion, or infection with HIV-1 (Table 1).

On the basis of the positive and negative RIBA-3™ results (i.e., excluding the RIBA-3™ indeterminate group), the predictive value of a positive HCV EIA-3 test result in this population was 18.8% (6 of 32, 95% CI = 7.0–36.4%) and the false-positive rate of the EIA-3 was 81.2% (26 of 32, 95% CI = 63.6–92.8%). We could not directly estimate the specificity of the HCV EIA-3 from these data because the 463 EIA-3 non-reactive samples were not tested by RIBA-3. However, if we assume that the EIA-3 non-reactive samples were truly negative, the specificity of the EIA-3 in this population was 94.7% (463 of 489, 95% CI = 92.3–96.5%).

The mean HCV EIA-3 OD value of the 6 RIBA-3™ confirmed samples was 2.3 (SD = ± 1.1, range = 1.0–3.5), which did not differ statistically from that of RIBA-3™ indeterminate samples (2.3, SD = ± 1.0, range = 0.75–3.5) or RIBA-3™ negative samples (2.6, SD = ± 1.0, range = 0.63–3.5). The frequency distribution of HCV EIA-3 OD values showed that repeatedly reactive specimens that were

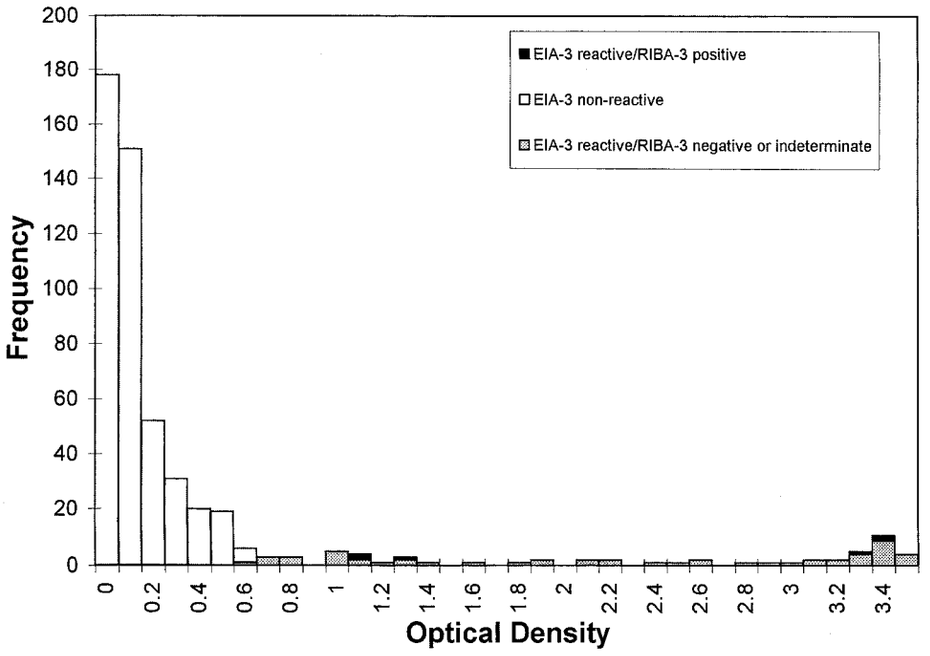

FIGURE 1. Distribution of hepatitis C virus enzyme immunoassay (EIA-3) optical density values of EIA-3 non-reactive samples, EIA-3 reactive/recombinant immunoblot assay-3 (RIBA-3™) negative or indeterminate sera, and EIA-3 reactive/RIBA-3™ positive sera.

not RIBA-3™ positive did not cluster at the cut-off value (Figure 1).

The pattern of reactivity by RIBA-3™ showed that only 1 RIBA-3™ positive serum reacted with all 4 bands, 3 sera reacted with c33c and c22p, and 2 reacted with c33c and NS5. Of the 21 RIBA indeterminate sera, 11 reacted against only c33c, 4 against only c22p, and 6 against only NS5 antigens. None of the 53 HCV EIA-3 repeatedly reactive sera had a positive RIBA-3™ SOD band, but a weak reaction to SOD (i.e., a less intense reaction than the Level I control band) was observed in 3 (50%) of 6 RIBA-3™ positive, 8 (38%) of 21 RIBA-3™ indeterminate, and 11 (42%) of 26 RIBA-3™ negative sera. In our laboratory, weak reactivity to the RIBA-3™ SOD band has been observed infrequently in other cohorts (2%, 4 of 207, of female U.S. intravenous drug users and 0.2%, 1 of 434, of hemophiliacs).

The IgG levels among 20 HCV EIA-3 negative sera (mean = 2,201 mg/dL, median = 2,210 mg/dL) were approximately 10% lower than that of 20 HCV EIA-3 false-positive sera (mean = 2,459 mg/dL, median = 2,465 mg/dL; $P = 0.05$ for comparison of the 2 means). The IgG levels in both groups were elevated relative to normal range values for U.S. adults (700–1,500 mg/dL).

Further investigation for the presence of HCV RNA by RT-PCR was carried out in RIBA-3™ positive and indeterminate samples. One (17%) of 6 RIBA-3™ positive sera and none of the 21 RIBA-3™ indeterminate specimens were positive for the presence of HCV RNA. The sole RT-PCR-positive specimen was also the only specimen with reactivity to all 4 RIBA-3 bands.

DISCUSSION

To our knowledge, this is the first report on the HCV seroprevalence in a general population sample in Tanzania. The observed prevalence rate (1.2%) was similar to that previously reported from Kenya (0.7–0.9%),[10,11] and together these studies suggest that HCV may be uncommon in East Africa. A low prevalence of HCV also has been reported among other populations in sub-Saharan Africa (Nigeria, 2%[3] and South Africa, 1.2%[12]), but higher estimates have been reported in Zaire (6%),[13] Cameroon (6%),[2] and Mozambique (3.2%).[14] However, results from these studies should be compared with caution because of differences in the anti-HCV testing methods and populations.

Of 53 HCV EIA-3 repeatedly reactive sera, only 6 (11.3%) were positive by HCV RIBA-3. Although false-reactivity with EIA systems has been reported in other studies,[11,14,15] the high discordance rate between the EIA-3 and RIBA-3™ results in our study led us to assess whether there was a pattern to the false-reactive HCV EIA-3 test results. We found no association between demographic characteristics and false positivity with the HCV EIA-3. Although other reports[14,16] have shown a significant correlation between low EIA ODs values and false EIA reactivity, this was not the case in our population (Figure 1).

False reactivity with EIA systems has been reported to occur in sera from populations with high levels of serum IgG that cross-react with the antigens used in the assay;[13,17] hypergammaglobulinemia has been observed in the general population in African countries related with endemic parasitic and infectious diseases.[18] Although the mean IgG levels of the Tanzanian samples were about 2 times higher than that expected for the general population of the United States, IgG levels were not clearly correlated with EIA-3 false reactivity: the mean IgG concentration in HCV EIA-3 false-positive sera was only about 10% higher than the mean IgG levels in EIA-3 non-reactive sera. Therefore, although elevated IgG levels may contribute to EIA-3 false reactions in

this population, differences in IgG levels cannot fully explain the high EIA-3 false-reactivity rate.

We also have concerns about the specificity of the RIBA-3 in this population because 42% of the sera tested with RIBA-3™ showed a weak reaction on the SOD band. Although the SOD reactivity was not strong enough to meet the manufacturer's criteria for an invalid assay, this unusually high prevalence of SOD band reactivity suggests that the assay might have detected interfering substances that adhere non-specifically to the solid phase. Another unusual feature among RIBA-3™ reactive samples is the low rate of 4 band reaction (only 1 of 6 samples). Finally, we found a poor correlation between RIBA-3™ positivity and the presence of HCV RNA. Only 1 (17%) of 6 RIBA-3™ positive samples were PCR positive, compared with 50–86% reported in HCV-infected populations in the United States.[4,19] Variant HCV, chance, false-positive RIBA-3™ results or improper serum storage are possible explanations for this poor correlation.[19,20] Specimens collection and transportation followed standardized procedures according to the study protocol. Sera were frozen under proper conditions in our repository and minimal manipulation has occurred. Therefore, it is unlikely that storage conditions have affected our results.

In conclusion, we found HCV to be uncommon both in urban and rural general populations in northwest Tanzania. We also found a higher rate of discordance between the EIA-3 and RIBA-3™ results than previously reported.[3,14] The low specificity and high levels of false-reactive results with the HCV EIA-3 among populations in tropical areas remain an important issue and might result from cross-reactivity with tropical pathogens or the presence of interfering substances that bind to the solid phase. The available HCV screening tests may be not suitable for routine serologic diagnosis of hepatitis C in African populations.

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